Variable chlorophyll fluorescence in response to water plus heat stress treatments in three coniferous tree seedlings

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Abstract: Effects of water and heat stress treatments on chlorophyll fluorescence of Chinese fir (*Cunninghamia lanceolata*), Masson pine (*Pinus massoniana*) and western redcedar (*Thuja plicata* D. Don) seedlings were monitored during a three-cycle stress period. It was shown that ratio of variable to maximal chlorophyll fluorescence (Fv/Fm) of these three species responded differently to water stress treatments. The Fv/Fm ratio of western redcedar decreased dramatically after water stress, while that of Chinese fir had only a slight reduction and that of Masson pine had no significant change. The experiment also showed that the Fv/Fm ratio of all three species differed significantly under heat stress treatments. Concerning three different water plus heat stress cycles, it was found that the Fv/Fm ratios of Chinese fir and Masson pine measured at the end of each water plus heat stress cycle were not significantly different. However, the Fv/Fm ratio of western redcedar was diminished significantly in response to an increase of stress time.

Keywords: Chinese fir; Chlorophyll fluorescence; Heat stress; Masson pine; Water stress; Western redcedar

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Introduction

Chlorophyll fluorescence is a non-destructive and rapid index used to assess in vivo plant photosynthetic activity (Vidaver *et al.* 1991; Schreiber *et al.* 1994), and a powerful tool for the assessment of plant physiological status (Hawkins *et al.* 1990). The effects of stresses such as water, heat, cold, air pollution and nutrient deficiency on fluorescence were well documented (Strand *et al.* 1987; Schreiber *et al.* 1993; Mohammed *et al.* 1995). Applications of chlorophyll fluorescence for evaluating physiological changes in forest trees include the assessment of freezing damage detection (Binder *et al.* 1996b), storage effect (Vidaver *et al.* 1991; Gillies 1993) and water stress (Vidaver et al 1991). In comparison to other seedling quality assessment methods, chlorophyll fluorescence technology is relatively quick to measure, non-destructive and compares favorably in terms of precision, accuracy, relevance and cost of measurement. It is also a more precise diagnostic tool for measuring stresses involving the photosynthetic system (Hawkins *et al.* 1990).

There are several chlorophyll fluorescence parameters that have been correlated to photosynthetic processes. One of the variables most widely studied with plants in general is Fv/Fm ratio, a quantitative measure of photochemical efficiency or optimal quantum yield of photosystem II (PS II). In particular, it is a good indicator of photoinhibition (Öquist *et al.* 1988). The Fv/Fm ratio has been used widely to monitor freezing damage, development of cold hardiness and effects of pollution. However, reports on its use for indicating water and heat stress are less common.

Chinese fir (*Cunninghamia lanceolata*) and Masson pine (*Pinus massoniana*) are two main species in southern China, accounting for about 90% of the planting programs in southern China each year. However, seedlings of the two species often suffer from severe drought and heat stress during summer. This study focuses on the possible utility of chlorophyll fluorescence as a tool to detect physiological changes when these species suffer water and heat stress. Prompt diagnosis of stress would allow nursery workers to take appropriate and effective measures to deal with drought and heat problems during seedling culture. As a non-subtropical tree species, western redcedar (*Thuja plicata* D. Don) was also chosen in these experiments for comparative purpose.

Materials and methods

Plant materials

Western redcedar (*Thuja plicata* D. Don) seeds (British Columbia Ministry of Forests seedlot No. B6542) were sown in D40 Deepot containers (10" in cell depth X2.5" in cell Diameter, Product of Stuewe & Sons inc. USA) on September 29, 2000. Masson pine (*Pinus massoniana* Lamb.) and Chinese fir (*Cunninghamia lanceolata* Hook.) seeds (both are from Sichuan province, China) were sown in the same kind of containers mentioned above on October 26, 2000. The growing medium in the containers was a

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mixture of 7: 2: 1 (v:v:v) peat moss: perlite: pasteurized mineral soil potting mix with Osmocote 13-13-13 added. Seedlings were maintained in a controlled environment greenhouse at the University of British Columbia campus, Vancouver Canada. Environmental conditions were shown in Table 1. The containers were watered regularly using clean water.

Table 1. Environmental conditions in greenhouse

Month	Temperature /°C			Humidity (RH%)			Photoperiod/h
	Minimum	Maximum	Average	Minimum	Maximum	Average	
October	14.7	25.2	20.8	10.1	99.8	55.4	16
November	19.0	24.1	20.8	5.3	79.5	43.7	16
December	20.0	25.2	21.4	0.5	78.0	33.8	16
January	20.0	24.8	21.5	8.6	71.2	41.6	16
February	20.0	24.7	21.6	0.9	62.4	30.3	16

Seedling treatment

After growing for about five months, seedlings of each species were randomly chosen to move into four controlled growth chambers (Conviron E15) to conduct water plus heat treatments from March 2 to April 7, 2001. Two of the growth chambers were set to maintain a high temperature regime at 28 ° C (15:00-9:00), at 35° C (9:00-15:00). The other two were set to maintain a more moderate temperature at 20 °C (15:00-9:00) and at 25 °C (9:00-15:00). Photosynthetic photon flux density in all four chambers was approximately 280-330 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Each chamber had 24-30 seedlings per species. Seedlings of each species were divided into three groups. Each group received a different watering treatment: dry (watered once every six days), medium (watered once every four days), wet (watered once every two days). The containers were irrigated to saturation each time. There were three rounds water stress cycles, and each cycle was twelve days.

Soil water potential estimations

At the end of each water stress cycle, soil samples were collected to estimate soil water potential. Soil moisture content of collected samples was measured using standard oven dry methods. The relationship between soil moisture content and soil water potential was established using a Wescor C52 thermocouple psychrometer chamber coupled with a HR33T microvoltmeter using the dewpoint method. Standard NaCl solutions were used for calibration. The resulting calibration curve was shown in Fig. 1. From this curve, a practical relationship between soil moisture content and soil water potential was derived (Table 2).

Chlorophyll fluorescence measurements

After each cycle of water stress treatments, the ratio of variable chlorophyll fluorescence to maximal chlorophyll fluorescence (Fv/Fm) of needles was measured with a portable fluorometer (Model OS-500 modulated fluorometer, Opti-Sciences, Inc. USA). Seedlings were acclimated to the dark for at least 20 min before the chlorophyll fluorescence measurements. The actinic photosynthetic photon flux density used for the chlorophyll fluorescence measurements was 91 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Table 2. The average soil moisture content and soil water potential measured at room temperature at the end of each water stress cycle

Water stress cycle	Soil moisture content (%)		Soil water potential /MPa	
	N	H	N	H
First cycle (12days)				
Wet (high)	61.68	46.23	-0.276	-0.450
Medium	50.01	32.37	-0.360	-2.244
Dry (low)	38.06	28.69	-1.077	-3.580
Second cycle (24 days)				
Wet (high)	54.10	43.07	-0.319	-0.600
Medium	48.01	30.52	-0.400	-2.842
Dry (low)	37.78	26.43	-1.117	-4.733
Third cycle (36 days)				
Wet (high)	56.55	42.71	-0.306	-0.623
Medium	45.36	31.86	-0.483	-2.395
Dry (low)	36.71	25.44	-1.280	-5.339

Notes: N: normal room temperature at 20 °C (15:00-9:00) and 25 °C (9:00-15:00); H: high temperature at 28 °C (15:00-9:00) and 35 °C (9:00-15:00).

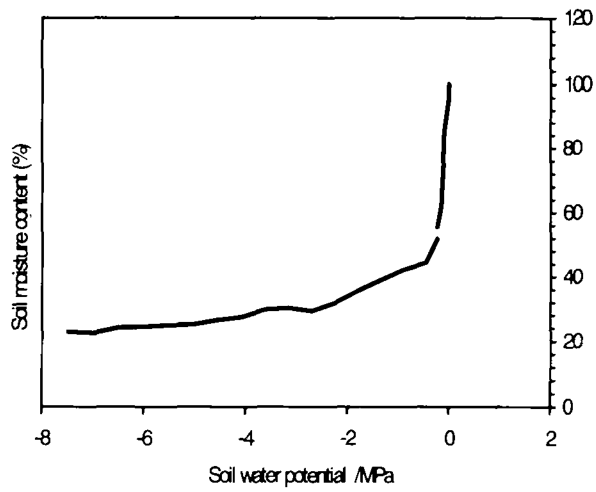

Fig. 1 The relationship between soil moisture content (%) and soil water potential.

The relationship can also be expressed as y= -174.287183 – 8.10838 x + 0.041404 x^2 – 0.000124 x^3 + 69.485401 $\sqrt{(x)}$; r^2=0.9912; x is soil moisture content; y is soil water potential.

Experimental design and statistical analysis

The experimental design was a randomized three-way plot. The three variables are water, temperature and growth chamber respectively. The water treatments had three levels: wet, medium and dry, while the temperature treatments had two levels: 28-35 °C and 20-25 °C. The growth chambers had two duplications. For each treatment, three seedlings were measured. There were a total of 36 seedlings per species (3 water stress treatments X 2 temperature treatments X 2 growth chambers X 3 seedling replicates).

The data was analyzed using SAS statistical software. A three-way ANOVA for the main effects of water, temperature and growth chamber on Fv/Fm ratios was performed and the variable difference was tested using Duncan's multiple range tests.

Results

Chlorophyll fluorescence under different water stress treatments

Chlorophyll fluorescence of the different species responded differently to water stress treatments (Table 3 and Fig. 2). The 12-day water stress treatment significantly influenced the Fv/Fm ratio of Chinese fir (p=0.0264, Table 3). The Duncan's multiple range testing showed that there was significant difference in Fv/Fm ratio of Chinese fir between the wet and dry treatments, and the medium and dry treatments. The wet and medium treatments did not differ. The Chlorophyll fluorescence of Masson pine was not significantly affected by any of the water stress treatments (p=0.8964, Table 3). For western redcedar, it was found that there were significant differences between wet and medium treatments as well as between wet and dry treatments (p=0.0001, Table 3), but there was no significant difference between the medium and dry treatments. Among the three species, western redcedar has the lowest Fv/Fm values (Fig. 2).

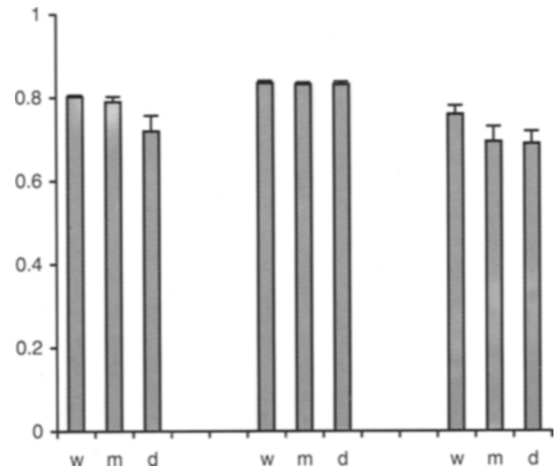

Fig. 2 The Fv/Fm ratio in response to three watering regimes lasting 12 days

w: watering once every two days; m: watering once every four days; d: watering once every six days; Species from left to right are Chinese fir, Masson pine and Western redcedar.

Table 3. Analysis of variance for Fv/Fm ratio in three conifer species after 12-day water plus heat stress treatments

Source of Variation	df	Chinese fir		Masson pine		Western redcedar	
		MS	*F*	MS	*F*	MS	*F*
Water treatment, [a]W	2	0.0264	3.50*	0.00003	0.11ns	0.0186	14.62***
Temperature, T	1	0.0454	6.02*	0.0035	12.01**	0.2930	230.44***
Growth chamber, GC	1	0.0202	2.67ns	0.0002	0.84ns	0.0031	2.41ns
Repeat, R	2	0.0038	0.50ns	0.0008	2.77ns	0.0020	1.58ns
Water and temperature interaction, W*T	2	0.0330	4.38*	0.00002	0.06ns	0.0059	4.65*

Notes: Significance levels are given as probability: ns, p>0.05; *p<0.05; **p<0.01. *** p<0.001.

Chlorophyll fluorescence under different heat stress treatments

The fluorescence of all three species was significantly affected by the heat stress treatments (Table 3). But the extent of Fv/Fm ratio changes is different. According to result of variance analysis, significant levels were as follows: Chinese fir (p=0.0209), Masson pine (p=0.0018) and western redcedar (p=0.0001). This means that the Fv/Fm ratio of western redcedar has the greatest reduction under heat stress treatments (Fig. 3).

Chlorophyll fluorescence at the different water plus heat stress treatments cycles

According to Fig. 4, it was found that the chlorophyll fluorescence of Chinese fir and Masson pine measured after different water and heat stress treatments cycles did not significantly differ. However, The Fv/Fm ratios of western redcedar decreased with repeated stress cycles.

Discussion

Both Chinese fir and Masson pine are subtropical species. Their seedlings often suffer severe heat and water stress. Nursery workers usually visually assess seedlings for damage by heat or water stress. These assessments are more or less subjective. Our experiment has detected some differences in Fv/Fm ratios of these two species following exposure to stressful conditions. The data indicate physiological changes occurring in these species in response to water plus heat stress. Chlorophyll fluorescence

has potential as a more simple and objective technique to estimate strain and the impact of water stress or heat stress.

Eastman and Camm (1995) observed that the photochemical efficiency of photosystem II in interior spruce seedlings and emblings decreased during water stress. We obtained similar results. Fig. 2 showed that the different species had different ability to tolerate water stress. Masson pine appeared to be the most tolerant to water stress because there were no significant differences in Fv/Fm ratios in this species under water stress treatment. Chinese fir, on the other hand, had significant differences in Fv/Fm among the different levels of water stress imposed, indicating greater susceptibility to this stress. Compared to these two subtropical tree species, the western redcedar obviously cannot tolerate as much water stress (Fig. 2). The Fv/Fm ratio was an index related to the photochemical efficiency of PS II. Reduced photosynthesis rate under water stress may result from the impairment of both leaf stomatal conductance and the ability of the mesophyll to fix available CO_2 other than photochemical efficiency of PS II (Epron *et al.* 1992, 1993; Genty *et al.* 1987; Kaiser 1987). Therefore, chlorophyll fluorescence may not change as much under water stress unless it is severe. Our experiment was consistent with this point of view. It depended on how long and how much the stress was conducted.

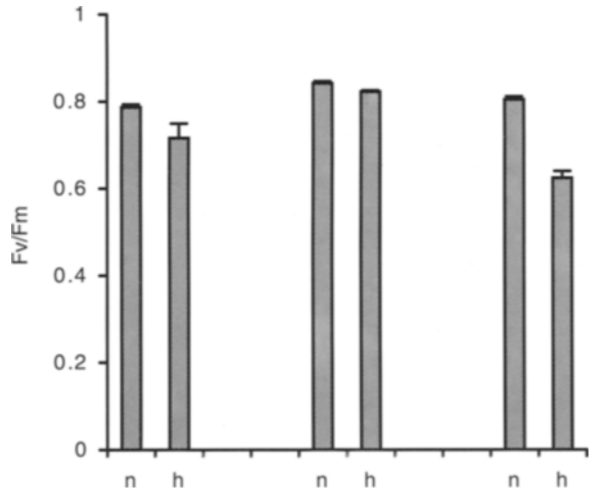

Fig. 3 The Fv/Fm ratio in response to temperature treatments lasting 12-day

n: normal temperature (20-25 °C); h: high temperature (28-35 °C); Species from left to right are Chinese fir, Masson pine and western redcedar.

Schreiber and Berry (1977) found that initial chlorophyll fluorescence rose as green cells become heat damaged at about 45 °C. Because Fv equals Fm-F0, the Fv/Fm ratio should decrease when seedlings suffered heat stress. Our experiment confirmed this finding (Fig. 3). Concerning the relative heat tolerance of the three species used, it seemed that Chinese fir and Masson pine were about the same, although the Fv/Fm ratios of both species had significant differences under different heat stress treatments. Western redcedar, however, was found to be much more susceptible to heat stress, as indicated by obviously reduction of Fv/Fm ratios after exposure to heat stress (Fig. 3).

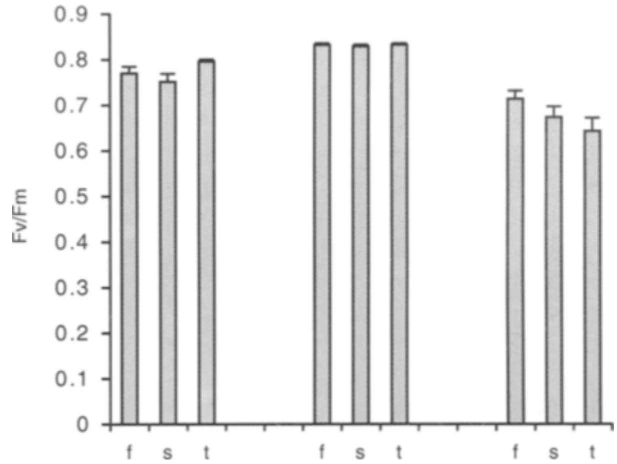

Fig. 4 The Fv/Fm fluorescence ratio in response to water plus heat stress treatmentsmeasured after three different stress cycle

f: measured after the first stress cycle; s: measured after the second stress cycle; t: measured after the third stress cycle; Species from left to right are Chinese fir, Masson pine and Western redcedar.

Table 4. The relative coefficient between chlorophyll fluorescence and seedling shoot length increment and shoot increment rate in three coniferous tree species

Items	Western redcedar		Masson pine		Chinese fir	
	r	*p*	*r*	*p*	*r*	*p*
Shoot length increment	0.7132***	0.0001	0.3214 ns	0.1094	0.4276*	0.0261
Increment rate (%)	0.7712***	0.0001	0.4338*	0.0268	0.2341 ns	0.2399

Notes: Significance levels are given as probability: ns, $p>0.05$; *, $p<0.05$; **, $p<0.01$. ***, $p<0.001$.

After three cycles of water and heat stress treatments, the chlorophyll fluorescence of both Chinese fir and Masson pine did not change significantly. This suggests that both species have a strong capacity to tolerate water and heat stress. Western redcedar, whose chlorophyll fluorescence decreased dramatically after three cycles of water plus heat stress treatments, was much susceptible to these combined stresses.

The interaction of water and heat stress has some effect on Chinese fir and Western redcedar, but not Masson pine (*P* values were 0.0566, 0.0184 and 0.9415 respectively, Table 3). High temperature and water stress may aggravate each other in Chinese fir and western redcedar. Havaux (1992) got a different result. He recognized that the stability

of photosystemII to heat was observed to increase strongly in leaves exposed to water stress treatments.

We also tried to establish the relationship between chlorophyll fluorescence and seedling shoot increment during the stress periods. The results showed that there was strong relationship between chlorophyll fluorescence and seedling shoot increment rate in western redcedar but not in both Chinese fir and Masson pine (Table 4). This is to further confirm that western redcedar did not grow well under water plus heat stress while Chinese fir and Masson pine did. The decrease of Fv/Fm ratios in western redcedar suggests its photosynthesis system was impaired.

Some yellowing of foliage was also observed during the experiment, particularly under heat stress. Yellowing is a visible indication of the possibility of severe damage to the photosynthetic system. Chlorosis of the sort observed usually means that photooxidation has happened and that chlorophyll concentrations have decreasing (Lamontagne et al. 2000).

BjÖrkman and Demming (1987) found that for a wide variety of C3 species, including tree species, an average value of 0.832 for the Fv/Fm ratio was typical of a well-functioning photosynthetic apparatus. This observation has been confirmed by Öquist and Wass's (1988) as well as others. This study showed that the average Fv/Fm ratios of Chinese fir, Masson pine and western redcedar under favorable conditions were 0.801, 0.823 and 0.827, respectively. These values were close to 0.832, consistent with a lack of stress in our control treatments.

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